



Some Studies on Reproduction in Goats

A thesis presented by

Haney Samir Mohamed Mohamed

(B.V.Sc., Fac. of Vet. Med., Cairo University, 2005)

(M.V.Sc. of Theriogenology, Fac. of Vet. Med., Cairo University, 2010)

For

The degree of **Ph.D** (Theriogenology)

Under the Supervision of

Prof. Mohamed A. I. El Sayed

Professor of Theriogenology
Fac. of Vet. Med., Cairo University, Egypt

Prof. Mostafa M. M. Abo-Ahmed

Professor of Theriogenology
Fac. of Vet. Med., Cairo University, Egypt

Prof. Gen Watanabe

Professor of Veterinary Physiology
Fac. of Agriculture
Tokyo University of Agriculture and
Technology, Tokyo, Japan

Dr. Aly M. A. Karen

Assistant Professor of Theriogenology Fac. of Vet. Med., Kafrelsheikh University, Egypt



Faculty of Veterinary Medicine

Department of Theriogenology

Approval Sheet

This is to approve that the thesis presented by:

Haney Samir Mohamed Mohamed

Cairo University, Faculty of Veterinary Medicine, Department of Theriogenology

Entitled "Some Studies on Reproduction in Goats"

For PhD Degree (Theriogenology) is approved by the Examination Committee:

A. A. Seldes

Holl A. Ismail

Prof. Dr. Abd El-Salam I. El-Azab

Prof. of Theriogenology

Faculty of Veterinary Medicine, Benha University

Prof. Dr. Adel Atia M. Seida

Prof. of Theriogenology

Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Mohamed A. I. El Sayed

Prof. of Theriogenology

Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Mostafa M. M. Abo-Ahmed

Prof. of Theriogenology

Faculty of Veterinary Medicine, Cairo University

M. M. Also Ahm

Op of Sala





SUPERVISION SHEET

Prof. Mohamed A. I. El Sayed

Professor of Theriogenology
Fac. of Vet. Med., Cairo University, Egypt

Prof. Mostafa M. M. Abo-Ahmed

Professor of Theriogenology

Fac. of Vet. Med., Cairo University, Egypt

Prof. Gen Watanabe

Professor of Veterinary Physiology,
Chairperson of Veterinary Department,
Fac. of Agriculture, Tokyo University of
Agriculture and Technology, Tokyo, Japan

Dr. Aly M. A. Karen

Assistant Professor of Theriogenology
Fac. of Vet. Med., Kafrelsheikh University, Egypt



CURRICULUM VITAE

PERSONAL DATA:

Full name Haney Samir Mohamed Mohamed Sayed Ahmed

Date of birth January 8, 1983

Nationality Egyptian
Marital status Married

Academic status Assistant lecturer

EMail: Haneyvet360@yahoo.com

Haneyvet360@cu.edu.eg
Haneysamir600@yahoo.com

Cell Phone: (202) 01003976362

Academic Qualifications:

(1) Bachelor of veterinary medical science (**BVSc**), Faculty of Veterinary Medicine, Cairo University, Egypt, with very good grade **May**, 2005.

(2) Master of veterinary medical science (MVSc), Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Egypt. Thesis entitled "Use of Ultrasonography in Studying Reproduction in Goats" February, 2010.

Scholarships awarded: Joint supervision scholarship Funded by Ministry of Higher-Education (MHE), Egypt for 2 years at Division of Animal Life Sciences, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology Supervised by Prof. Dr. Gen Watanabe, from March 2013 to March 2015.

<u>Google scholar:</u> http://scholar.google.com/citations?user=M3pAPyAAAAJ&hl=en Research gate:

http://www.researchgate.net/profile/Haney Samir

Cairo University scholar: http://scholar.cu.edu.eg/?q=pcr/publications

Languages and computer skills

o English: Good writing and Speaking

o Internet Based TOEFL IBT, Egypt, 2012

o International Computer License ICDL, Ministry of communications and information technology, Egypt, 2011.

General abstract

This study was carried out in 4 experiments for studying important points associated with reproductive performance in goats.

First experiment aimed to investigate the incidence, timing and factors associated with embryonic/fetal losses (EFL) in 151 goats of different breeds [Zaraiebi, Damascus, and Cross goats]. All goats were examined by B-mode ultrasonography to monitor EFL during different stages of gestation. Results revealed a high EFL% between D20-23 to D47-54 (19.61 %) compared to the period >D47-54 to birth (11.76 %). A high EFL% was observed in Zaraiebi goats compared to others. The goats that were synchronized by P4 sponge, as well as, artificially inseminated showed a higher EFL % compared to goats with spontaneous estrus, and bred by natural mating, respectively.

The 2nd experiment goaled for evaluating testicular blood flow at supratesticular artery (STA) in 8 male Shiba goats by color Doppler ultrasonography after administration of either GnRH or hCG. Results indicated an increase of testicular blood flow in both groups. However, this increase was significantly higher and earlier in hCG group (1 hour) than that in GnRH group (2 hours).

The 3^{rd} experiment aimed to identify whether inhibin hormone has a role in testicular hemodynamic in goats. Therefore, 9 adults Shiba bucks were injected with 10 ml of either inhibin antiserum (INH group; n = 5) or normal castrated goat serum (NGS group; n = 4). Results revealed significant decreases in the resistive index (RI) values of the STA and marginal testicular artery (MTA) in the INH group compared to those in the NGS group, which indicate an increase of testicular blood flow following immunization.

Finally, **the 4th experiment** aimed to investigate the expression and localization of kisspeptin in the testes of Shiba goats by real time PCR, and immunohistochemistry, respectively. Results revealed testicular expressions of mRNA encoding KISS1 and immunoreactivity of kisspeptin in Leydig cells. These expressions were higher in the postpubertal goats compared with that in the prepubertal goats.

Key words:

Color Doppler, Embryonic/fetal losses, Goats, Immunohistochemistry, Kisspeptin, PCR, Testicular blood flow.

DEDICATION

To all members in my family (my mother, father, brothers and my sister), for their support, guidance and good education. Without your love, I could not be this far.

To my beloved wife (**Faten**), and my sweet son (**Yassin**), for your patience, help, love, support and understanding throughout my study. Without you, I could not overcome both large and small obstacles along my study.

ACKNOWLEDGEMENTS

First thanks for the merciful GOD who gave me strength and support to learn this new technology and achieve this work.

I would like to express my great gratitude to my promotor Prof. Dr. Mohamed A.I. El Sayed, prof. of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, for his planning, kind supervision, valuable criticism and continuous encouragement.

I would like also to express my sincere appreciation to my promotor Prof. Dr. Gen Watanabe, prof. of Veterinary Physiology, Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Tokyo, Japan, for his kind supervision, dedication, worthful advices, and generous encouragement and help throughout my work.

I would like also to extend my warmest appreciation to Prof. Dr. Mostafa M.M. Abo-Ahmed, prof. of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, for kind supervision, and continuous encouragement.

Great gratitude is being expressed to Dr. Aly M. A. Karen, assistant Professor of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt, for his supervision, constructive criticism and guidance in writing of the thesis.

I would also like to thank, and extend my warmest appreciation to Dr. Kazuyoshi Taya, Dr. Kentaro Nagaoka, and all my colleagues in Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology, Tokyo, Japan, for their support, help, cooperation, friendship and encouragement during this study.

I am greatly indebted to Dr. **Kazuaki Sasaki**, Laboratory of Veterinary pharmacology, Tokyo University of Agriculture and Technology, Tokyo, Japan, for his great help and offering color Doppler ultrasound machine.

Great thanks to all my professors and colleagues in the Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt, for teaching and help.

I would like to thank my family, for their everlasting encouragement and Support in all my activities.

I would also like to thank Station of Sheep and Goat Research (Sakha) belonging to Animal Production Research Institute, Agriculture Research Centre, Kafr El-Sheikh governorate, Egypt for performing the first experiment in this study.

Grateful thanks to Ministry of Higher-Education (MHE), Egypt for funding of a Joint supervision scholarship in the period from March 2013 to March 2015.

Acknowledgement for providing antisera during the practical part:

We are also grateful to **Dr. A.F. Parlow** (National Institute of Diabetes and Digestive and Kidney Diseases, Beltsville, MD) for providing the RIA materials for ovine FSH and LH, Dr. **G.D. Niswender** (Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO) for providing antisera to estradiol-17 β (GDN 244) and testosterone (GDN 250), and Dr. **Y. Mori** (University of Tokyo, Bunkyo-ku, Tokyo, Japan) for antiserum to ovine LH (YM-18). Additionally, we are grateful to Dr. **H. Okamura** (Laboratory of Neuroendocrinology, National Institute of Agrobiological Sciences, Ibaraki, Japan) for antiserum to kisspeptin (C2). We also express our gratitude to Dr. **Kei-ichio Maeda** (Department of Veterinary Medical Sciences, The University of Tokyo, Tokyo, Japan) for his instrumental advice.

DECLARATION

I hereby declare that this thesis submitted by me to Cairo University for the degree of PhD, has not previously been submitted for a degree to any other university. I further cede copyright of the thesis in favour of Cairo University, Egypt.

Haney Samir Mohamed Mohamed

June, 2015

LIST OF ABBREVIATIONS

ABBREVIATIONS	IDENTIFICATION
I.M	Intramuscular
iv	Intravenous
IU	International unit
TAU	Transabdominal ultrasonography
TRU	Transrectal ultrasonography
MHz	Mega hertz
ng/ml	Nanogram/millilitre
EFL	Embryonic and fetal losses
EIA	Enzyme immuno assay
SEM	Standard error mean
CL	Corpus luteum
r	Pearson correlation coefficient
P	P-value
AI	Artificial Insemination
ANOVA	Analysis of Variance
eCG	Equine Chorionic Gonadotrophin
FGA	Flurogestone Acetate
PGF2α	Prostaglandin F2α
IFN tau	Interferon tau
D	Day
P4	Progesterone
°C	Celsius degree
EL vs FL	Embryonic loss versus fetal loss
S1 vs S2	EFL at D20-23 to D47-54 versus >D47-54 to kidding
hr	hour
gr	gravity
sec	second
cm	centimeter
gm	gram
Kg	Kilogram

rpm	Revolutions per minute
PMSG	Pregnant mare serum gonadotropin
oTP-1	Ovine trophoblast α interferons
bTP-1	Bovine trophoblast α interferons
cTP-1	Caprine trophoblast α interferons
PRID	Progesterone-releasing intravaginal device
RIA	Radioimmunoassay
L * W * H * 0.71	Length x Width x Heigh x 0.71
Pg/ml	Picogram/millilitre
Т	Testosterone
E2	Estradiol
INH	Inhibin
FSH	Follicle Stimulating Hormone
GnRH	Gonadotrophin Releasing Hormone
hCG	Human Chorionic Gonadotrophin
LH	Luteinizing Hormone
oFSH	Ovine Follicle Stimulating Hormone
NSS	Normal sheep serum
NRS	Normal rabbit serum
ASGG	Anti-sheep gamma globulin
ARGG	Anti-rabbit gamma globulin
IgG	Immunoglobulin G
mM	Millimole
M	Mole
Ab	Antibody
PEG	Polyethylene glycol
IHH	Idiopathic Hypogonadotropic Hypogonadism
IF	Interstitial fluid
VEGF	Vascular endothelial growth factor
PBS	Phosphate buffer saline
BSA	Bovine serum albumin
ВРН	Benign prostatic hyperplasia
NGS	Normal goat serum

PSV	Peak systolic velocity
EDV	End diastolic velocity
RI	Resistive index
PI	Pulsatility index
STA	Supratesticular artery
MTA	Marginal testicular artery
TV	Testis volume
m.PSV	Peak systolic velocity of the marginal testicular artery
m.EDV	End diastolic velocity of the marginal testicular artery
TAMAX	Time average maximum blood velocity
mRNA	Messenger ribonucleic acid
RT.PCR	Real time polymerase chain reaction
ActRII	Activin receptor II
m.RI	Resistive index of the marginal testicular artery
m.PI	Pulsatility index of the marginal testicular artery
TGF β type III	transforming growth factor beta receptor III
c-DNA	Complementary DNA
KISS1	Kisspeptin mRNA
GPR54	G protein- coupled receptor
ARC	Arcuate nucleus
AVPV	Anteroventral periventricular nucleus
КО	Knockout
P450scc	cytochrome P450 side-chain cleavage
3βHSD	3beta-hydroxysteroid Dehydrogenase
P450c17	cytochrome P450, 17alpha-hydroxylase/17, 20 lyase
P450arom	cytochrome P450 aromatase
C2 antiserum	Antikisspeptin antibody
PBST solution	0.05 mole of PBS+0.3 % Triton X-100

LIST OF CONTENTS

CONTENTS	Page
DEDICATION	Ι
ACKNOWLEDGEMENTS	
DECLARATION	V
LIST OF ABBREVIATIONS	VI
LIST OF CONTENTS	IX
LIST OF FIGURES	XI
LIST OF TABLES	XIV
GENERAL INTRODUCTION	1
REVIEW OF LITERATURE	6
CHAPTER 1: Monitoring of embryonic/fetal losses in different	40
breeds of goats using real time B- mode ultrasonography.	
1.1.INTRODCUTION	40
1.2.MATERIAL AND METHODS	42
1.3.RESULTS	50
1.4.DISCUSSION	64
1.4.DISCUSSION	64
1.5.CONCLUSION	70
CHAPTER 2: Effect of a single injection of gonadotropin-	
releasing hormone (GnRH) and human chorionic gonadotropin	
(hCG) on testicular blood flow measured by color Doppler	
ultrasonography in male Shiba goats. 2.1.INTRODCUTION	71
2.2.MATERIAL AND METHODS	73
2.3.RESULTS	82
2.4.DISCUSSION	96
2.5.CONCLUSION	102
CHAPTER 3: Can passive immunization against inhibin	103
improve testicular blood flow that measured by color Doppler	
ultrasonography in male Shiba goats?	
3.1.INTRODCUTION	103
3.2.MATERIAL AND METHODS	106
3.3.RESULTS	115

3.4.DISCUSSION	132
3.5.CONCLUSION	137
CHAPTER 4: Changes in the testicular expression of KISS1	
mRNA and kisspeptin immunoreactivity from the prepubertal to	
postpubertal goats.	
4.1.INTRODCUTION	138
4.2.MATERIAL AND METHODS	140
4.3.RESULTS	145
4.4. DISCUSSION	151
4.5.CONCLUSION	153
GENERAL DISCUSSION	154
GENERAL SUMMARY	161
REFERENCES	164
VALIDATION REPORT	1
ARABIC SUMMARY	i